The Impact of Supplemental Dietary Methionine Sources on Volatile Compound Concentrations in Broiler Excreta

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ABSTRACT The impact of different Met sources on broiler fecal odor volatiles was determined by evaluating the types of sulfur compounds produced in broiler excreta. Two experiments were conducted using straight-run broiler chicks randomly distributed in battery cages, with 3 replicate pens of 16 birds each. The treatment groups were 1) dry Met hydroxy analogue (dry MetHA), 2) sodium methioninate aqueous solution (NaMet), 3) liquid Met hydroxy analogue (Liq MetHA), 4) D,L- Met, and 5) no supplemental Met (control group). The Met activities of each Met source were 52, 45.9, 88, and 98%, respectively. All diets were formulated to contain either 0.8% (experiment 1) total Met activity or 0.5% Met activity in the starter and 0.38% Met activity in the grower (experiment 2) (except the control group, 0.35% Met activity), but otherwise met NRC nutrient requirements (NRC, 1994). Diets were fed ad libitum from d 1 to 6 wk of age. There were no significant differences in BW among the treatments. All excreta were collected in litter pans lined with aluminum foil. In experiment 1, at wk 6, broiler excreta were collected for a 24-h period, and 4.5 g of broiler excreta from each treatment group was collected into 15-mL headspace vials. Samples were analyzed by gas chromatography/mass spectrometry (GC/MS). The volatile sulfur compounds that were identified and quantified in the broiler excreta were H_2S, carbonyl sulfide (COS), methyl mercaptan (CH_3SH), dimethyl disulfide (CH_3SSCH_3), and dimethyl trisulfide (CH_3SSSCH_3). The NaMet treatment group had significantly higher concentrations of H_2S, COS, and CH_3SSCH_3 compared with all other treatment groups. The Liq MetHA group had significantly lower concentrations of H_2S, COS, CH_3SH, and CH_3SSCH_3 compared with the other treatment groups. The dry MetHA group significantly had the highest concentration of CH_4SH. The D,L-Met treatment group had the significantly highest concentration of CH_3SSSCH_3 and the lowest concentration of H_2S. The control group had the significantly lowest concentrations of CH_3SH, CH_3SSCH_3, and CH_3SSSCH_3 compared with the other treatment groups. In experiment 2, at wk 6, an electronic nose was used to evaluate 15 air samples per treatment group. In addition, 15 air samples (containing 6 to 8 L of air in a Tedlar bag, 3 samples per treatment group) were collected for odor evaluation by a sensory panel. Electronic nose sensor data revealed that volatile compounds in broiler excreta from the control group were significantly different from the other 4 treatment groups. Evaluation of the air samples by a sensory panel determined that there was a statistically significant difference in odor threshold detection between the control group and the other treatment groups. The dilutions to threshold of control group, NaMet, dry MetHA, Liq MetHA, and D,L-Met were 350, 492, 568, 496, and 526 odor units, respectively. These findings demonstrate that dietary Met sources significantly influenced odorous volatile concentrations in broiler excreta.

(*Key words*: broiler excreta, electronic nose, methionine, odor, sensory panel)

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INTRODUCTION

Over 8 billion broilers are reared each year for meat production in the United States. The grow-out period for commercial broilers is typically 6 wk, ending with a final BW of 2 kg. During this period, each bird will excrete approximately 3 kg of manure, which yields a total of 24 billion kg of manure produced in the US annually (USDA/NASS, 2002). Most odor nuisance re-

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Abbreviation Key: MetHA = methionine hydroxy analogue; GC/FDP = gas chromatography with sulfur selective flame photometric detection; GC/MS = gas chromatography/mass spectrometry; ISUOL = Iowa State University Olfactometry Laboratory; Liq MetHA = liquid methionine hydroxy analogue; NaMet = sodium methioninate aqueous solution.

ports concerning poultry farms are due to poultry manure. Before odor prevention and control can be implemented in poultry waste management, the cause of the odor must be established (Mackie et al., 1998). O'Neill and Phillips (1992) have indicated that 6 of the 10 compounds with the lowest odor thresholds in livestock manure contain sulfur, suggesting that these compounds may be the most likely to be associated with odor nuisance reports. The primary source of sulfur in broiler feces is dietary sulfur amino acids, with Met being the major dietary sulfur amino acid.

Met is biochemically related to odorous compounds (methyl mercaptan, hydrogen sulfide, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, and carbonyl sulfide) because it serves as the initial product or precursor for the formation of these compounds through microbial decomposition (Kadota and Ishida, 1972).

Microbes utilize Met and form intermediate compounds such as methyl mercaptan and ketobutyrate. Methyl mercaptan can be further utilized to form hydrogen sulfide and methyl alcohol. The following diagram illustrates this process (Clanton and Schmidt, 2000):

$$\begin{gathered} CH_3SCH_2CH_2CHNH_2COOH + H_2O \\ \text{methionine + water} \\ \rightarrow CH_3SH + NH_3 + CH_3CH_2COCOOH \\ \text{methyl mercaptan + ketobutyrate} \end{gathered} \quad [1]$$

$$\begin{gathered} CH_3SH + H_2O \\ \text{methyl mercaptan + water} \\ \rightarrow CH_3OH + H_2S \\ \text{methyl alcohol + hydrogen sulfide} \end{gathered} \quad [2]$$

Dimethyl disulfide is the oxidation product of methyl mercaptan. Dimethyl trisulfide is produced through the oxidation of hydrogen sulfide and methyl mercaptan (Zahn et al., 2001). Met also serves as a precursor to Cys. This is important because Cys is a major component of poultry feathers. Microbes can utilize Cys to form volatile compounds such as carbonyl sulfide (Banwart and Bremner, 1975). Microbes also utilize Met to produce such derivatives as α-ketobutyrate, ammonia, methyl mercaptan, s-methyl-cysteine, and s-methyl-methionine. s-Methyl-methionine is the main precursor for the formation of dimethyl sulfide (Lomans et al., 2002).

Previous work in our laboratory (Chavez et al., 2001) using an electronic nose has shown that supplemental Met sources in poultry diets affect the production of odorants in broiler excreta. An electronic nose is an instrument containing an array of electronic chemical sensors coupled to a pattern recognition system capable of recognizing simple or complex odors (Gardner and Bartlett, 1994). The electronic nose is equipped with a probe, which allows air samples to be collected at set intervals. As air is drawn inside the instrument, individual sensors respond differently to different groups of volatile compounds in the air sample. The sensors produce distinct patterns for particular odors, which are recognized by the pattern recognition system. These distinct patterns are fingerprints of the odor. The responses or patterns are produced by a change in electronic resistance of the sensor, which is recognized by the pattern recognition system (Hobbs et al., 1995). A greater change in the polymer sensor's resistance indicates a higher electronic nose reading. A higher electronic nose reading demonstrates that a greater quantity of volatile compounds is being absorbed by the polymer sensor (Li, 2000). However, in the previous study, the electronic nose was not capable of identifying differences in specific individual volatile compounds in broiler excreta (Chavez et al., 2001). With the use of gas chromatography-mass spectrometry (GC/MS), individual volatile compounds (specifically sulfur volatile compounds) can be separated and identified in broiler excreta. Previous studies have used this analytical method to identify specific volatile compounds from different livestock manure (Yasuhara, 1987; Hobbs et al., 1995).

A previous study (Chavez et al., 2001) by our laboratory used higher Met levels than are recommended by the National Research Council (NRC, 1994). Starter and grower diets were formulated to contain 0.80% Met activity for all treatment groups except the control group (0.35% Met activity for both diets). These diet formulations were used for experiment 1 of this current study. Broiler excreta produced at this Met level were evaluated by gas chromatography with sulfur-selective flame photometric detection (GC/FPD), and assignments were confirmed with GC/MS.

The NRC (1994) recommends 0.50 and 0.38% for both starter and grower diets, respectively. In the current study (experiment 2), Met activity levels for both starter and grower diets were based on NRC (1994) recommendations. Broiler excreta produced at this Met level were evaluated by the electronic nose and human olfactory panel.

A human olfactory panel uses the nose with an olfactometer, which dilutes odor samples with odor-free air. This sensory measurement (olfactometry) is the most common for odor sample evaluation of odor detection thresholds. Odor threshold concentration is expressed as the number of dilutions of odor-free air required to achieve this threshold concentration (Gostelow and Parsons, 2000). Olfactometry measurement is dependent on the test procedure design. A number of variables could affect olfactometry measurements. Some key variables are the test procedure, differing sensitivities of observers, and the method of calculation of odor concentration of an air sample (Dravnieks and Jarke, 1980). This procedure has been utilized to measure odor concentrations in livestock manure (Powers and Faust, 1998, Misselbrook et al., 1997).

The objectives of this study were to 1) evaluate differences in volatile sulfur compound concentrations in broiler excreta with different Met treatments, 2) determine if the lower Met activity levels produced detectable differences in volatile odor production, and 3) evaluate

odor detection thresholds of broiler excreta air samples from the different treatment groups.

MATERIALS AND METHODS

Design

Two experiments were conducted using straight run broiler chicks raised in battery cages at Texas A&M University Poultry Science Center. All broiler chicks used in our experiments were randomly distributed into 5 treatment groups consisting of 3 replicate pens of 16 birds each. Each pen contained an individual litter pan. The treatment groups were 1) dry Met hydroxy analogue (dry MetHA), 2) sodium methioninate aqueous solution (NaMet), 3) liquid Met hydroxy analogue (Liq MetHA), 4) D,L-Met, and 5) no supplemental Met (control group). The Met activities of each source were 52, 45.9, 88, and 98%, respectively. Corn-soybean based diets were formulated to contain 3,135 kcal of ME/kg in the starter ration, and 3,200 kcal of ME/kg in the grower ration. The starter and grower rations were formulated to contain 23 and 21% CP, respectively. For experiment 1, starter and grower diets were formulated to contain 0.80% total Met activity except for the control group, which had 0.35% Met activity (Table 1). However, analysis of the experiment 1 control diet for Met showed that it contained 0.26 and 0.20% for starter and grower diets, respectively (Table 2). For experiment 2, the starter and grower diets were formulated to contain 0.50 and 0.38% Met activity except for the control group, which had 0.35% Met activity for both diets (Table 3). However, analysis of the experiment 2 control diets for Met showed the diets contained 0.21 and 0.23% Met for the starter and grower diets, respectively (Table 2). This Met concentration was lower than the diet formulation was calculated to contain. This difference could be attributed to variation in protein concentration in feed ingredients (corn and soybean meal) containing lower Met activity than was utilized in the computer program for diet formulations. All other nutrient requirements met or exceeded NRC recommendations (NRC, 1994). All feeds were mixed at Texas A&M University Poultry Science Center. Diets were fed ad libitum from d 1 to 42, and all birds were weighed weekly.

Quantification of Volatile Sulfur Compounds in Poultry Feces

In experiment 1, all poultry feces for all treatments were collected for 24 h from broilers at 6 wk of age. Fresh poultry fecal matter (0.750 g) was transferred into a glass 15-mL headspace vial containing 1.50 mL of 8 m*M* KCl and an octagonal microstir bar.[2] The vial was immediately capped with a screw cap fitted with a Teflon coated silicone septa, and exactly 100 μL of 6 *M* HCl was injected into the vial to stop microbial activity and increase volatility of sulfide and mercaptan compounds. The vial was incubated on a stir-plate water bath (50°C) for 15 min, and the volatile compounds were collected from the headspace by solid phase micro extraction using a 2-cm 50/30 μm DVB/Carboxen/PDMS stable flex fiber assembly. The fiber was exposed in the headspace of the vial for 10 min and then removed and injected into an Agilent 6890 gas chromatograph[3] equipped with a sulfur-selective flame photometric detector (GC/FDP) and electron impact ionization mass spectrometer (GC/MS).[3] Volatile compounds were desorbed from the fiber using a splitless injector temperature at 240°C and were separated on a 0.32-mm × 30-m × 4-μm film SPB-1 sulfur fused silica column,[4] according to methods previously described (Bulletin 876;[4] Mestres et al., 1999). Identification of compounds in the poultry excreta was based on retention times in comparison to authentic chemical standards purchased for gas-phase[5] [H_2S, carbonyl sulfide (COS), methyl mercaptan (CH_3SH)] and liquid-phase[2] [dimethyl disulfide (CH_3SSCH_3), dimethyl trisulfide (CH_3SSSCH_3)] and was confirmed by electron-impact ionization mass spectrometry as previously described (Zahn et al., 2001). For compounds with boiling points below room temperature (H_2S, COS, CH_3SH), known static gas-phase concentrations from permeation tube emission sources were transferred into empty headspace vials to calibrate the SPME device for gas-phase measurements. Gas-phase concentrations were converted to manure headspace equilibrium concentrations using published Henry's law constants for H_2S (0.087 mol/kg × bar), COS (0.022 mol/kg × bar), and CH_3SH (0.26 mol/kg × bar) at 298.2°K. For CH_3SSCH_3 and CH_3SSSCH_3, direct manure headspace equilibrium concentrations were determined by internal standard additions into poultry manure samples.

Electronic Nose Study

In experiment 2, at 6 wk of age, feces for all treatments were collected for 24 h in litter pans lined with aluminum foil. Litter pans were individually transferred to a separate room for odor volatile analysis using a Cyranose 320 electronic nose.[6] The electronic nose contained a sensor array of 32 semiconducting polymer-type sensors labeled 0 through 31. Air samples were collected from 1 to 5 cm away from the broiler excreta.

Individual Met treatment groups were tested separately. The electronic nose was used to capture 5 air samples from various locations near fresh feces for each pan of broiler excreta, resulting in a total of 15 air samples from each treatment group. The electronic nose was recalibrated by drawing in fresh air before testing each treatment group. The output from each of the 32 sensors

[2]Fisher Scientific, Chicago, IL.
[3]Model 5973N, Agilent Technologies, Inc., Palo Alto, CA.
[4]Supelco, Bellefonte, PA.
[5]Kin-Tek Laboratories, Inc., LaMarque, TX.
[6]Model A-32S, Cyrano Sciences, Inc., Pasedena, CA.

TABLE 1. Composition of starter[1] and grower[2] rations for experiment 1

Feed ingredient and diet	Control[3]	D,L-Met[4]	Dry MetHA[4]	NaMet[4]	Liq MetHA[4]
	Diet (%)				
Starter ration					
Corn	53.43	53.68	51.69	51.53	52.38
Soybean meal 48	38.06	37.45	38.36	38.39	38.24
Fat, animal and vegetable blend	4.63	4.51	5.19	5.24	4.97
Limestone ground	1.68	1.68	1.68	1.68	1.68
Monodicalcium PO_4	1.54	1.55	1.54	1.54	1.54
Salt	0.36	0.36	0.36	0.18	0.36
Trace minerals premix[5]	0.05	0.05	0.05	0.05	0.05
Vitamin Premix[6]	0.25	0.25	0.25	0.25	0.25
Supplemental Met	0	0.46	0.87	1.13	0.52
Grower ration					
Corn	61.42	61.70	59.54	59.37	60.32
Soybean meal 48	30.47	29.82	30.80	30.83	30.66
Fat, animal and vegetable blend	4.43	4.30	5.04	5.10	4.79
Limestone ground	1.45	1.45	1.45	1.45	1.45
Monodicalcium PO_4	1.59	1.60	1.59	1.59	1.59
Salt	0.34	0.34	0.34	0.14	0.34
Trace minerals premix[5]	0.05	0.05	0.05	0.05	0.05
Vitamin premix[6]	0.25	0.25	0.25	0.25	0.25
Supplemental Met	0	0.50	0.94	1.22	0.55

[1]Diet contained 3,135 kcal of ME/kg and 23% CP.

[2]Diet contained 3,200 kcal of ME/kg and 20% CP.

[3]Total Met activity in diet (0.35%).

[4]Total Met activity in diet (0.80%). Dry MetHA = dry Met hydroxy analogue; NaMet = sodium methioninate aqueous solution; Liq MetHA = liquid Met hydroxy analogue.

[5]Trace mineral premix added at this rate yielded 149.6 mg of Mn, 125.4 mg of Zn, 16.5 mg of Fe, 1.7 mg of Cu, 1.05 mg of I, 0.25 mg of Se, a minimum of 6.27 mg of Ca, and a maximum of 8.69 mg of Ca/kg of diet. The carrier was Ca carbonate, and the premix contains less than 1% mineral oil.

[6]Vitamin premix added at this rate yielded 11,023 IU of vitamin A, 3,858 IU of vitamin D, 46 IU of vitamin E, 0.0165 mg of B_{12}, 5.845 mg of riboflavin, 45.93 mg of niacin, 20.21 mg of D-pantothenic acid, 477.67 mg of choline, 1.47 mg of menadione, 1.75 mg of folic acid, 7.17 mg of pyroxidine, 2.94 mg of thiamin, and 0.55 mg of biotin/kg of diet. The carrier was ground rice hulls.

was averaged over a 10-s interval and recorded as a single data point for each individual sensor.

Broiler excreta were also evaluated for moisture content in experiment 2. Excreta were collected for 24 h and placed in aluminum foil cups. The aluminum foil cups were then weighed and placed in a drying oven at 100°C for 24 h and then reweighed to calculate moisture loss.

Olfactometry Laboratory Odor Evaluation Procedure

In experiment 2, air samples were taken at wk 6 to analyze for odor detection threshold by a sensory panel trained to evaluate odor detection threshold of air samples. Detection threshold is the minimum concentration at which an odor can be detected. All samples were collected in individual 10-L Tedlar[7] bags by a universal pump[8] and a Vac-U-Chamber.[8] A Tedlar tube was used to draw the air into the sample bags. The tube was placed the same distance from the excreta as previously described in the electronic nose study. All air samples range from 2 to 3 min for collection. A total of 15 air samples (containing 6 to 8 L of air in a Tedlar bag, 3 samples per treatment group) were collected for odor evaluation by a sensory panel. All air samples were immediately sent to the Iowa State University Olfactometry Laboratory (ISUOL) and analyzed within 24 to 48 h of collection. All air samples were evaluated for odor detection threshold by a trained panel and for hydrogen sulfide concentration by a Jerome hydrogen sulfide analyzer.[9]

The olfactometry laboratory odor evaluation procedure consisted of sensory panelists given 3 stimulus presentations in random order (Triangular Forced Choice Method, ISUOL, 2001). Out of the 3 presentations, 1 contained the diluted sample, and the remaining 2 were fresh air. The panelist was required to identify which presentation contained the odorous sample. The sample was delivered through the olfactometer at 20 L/min for a sniffing time of 3 s. The sample was primed for 5 s before presentation. Purging time between samples was 5 s. Once the odor was sensed, each panelist selected which of the 3 presentations contained the diluted sample and indicated detection on the olfactometer. Following 1 correct detection, the panelist was required to have an additional correct detection at the next highest dilution (ISOUL, 2001).

[7]Plastic Tedlar bag, ISUOL, Ames, IA.

[8]Pump model 224-44XR and SKC Vac-U-Chamber, SKC, Inc., Eighty Four, PA.

[9]Model 631-X, Arizona Instrument, Tempe, AZ.

TABLE 2. Analyzed nutrients of experiment 1 and 2 control feed samples

Analyzed nutrients[1]	Experiment 1		Experiment 2	
	Starter	Grower	Starter	Grower
	(%)			
Dry matter	89.39	89.58	88.74	90.09
Protein	19.63	18.48	16.66	17.05
Asx	1.54	1.10	0.95	1.07
Glx	4.48	4.51	4.27	4.15
Ser	1.01	0.98	0.93	0.90
His	0.53	0.54	0.42	0.49
Gly	0.77	0.76	0.64	0.66
Thr	0.73	0.71	0.63	0.67
Ala	0.98	0.95	0.87	0.88
Arg	1.61	1.53	1.29	1.31
Tyr	0.52	0.42	0.42	0.42
Val	0.98	0.91	0.72	0.84
Met	0.26	0.20	0.21	0.23
Phe	1.07	0.99	0.92	0.92
Ile	0.91	0.83	0.67	0.76
Leu	1.81	1.68	1.58	1.60
Lys	1.28	1.25	1.07	1.11
Pro	1.16	1.11	1.06	1.05

[1]Analyses were performed by Texas A&M University Protein Chemistry Laboratory (Cys and Trp were not quantified in HCL hydrolysis assay).

Panelists were tested and trained before participation in the olfactometry lab. Panelists were considered fully trained when samples were given and the standard deviation of the detection threshold values was below 0.5. All sample evaluation was performed at one time, and panelists were kept separate from the olfactometer room

TABLE 3. Composition of starter[1] and grower[2] rations for experiment 2

Feed ingredient and diet	Control[3]	D,L-Met[4,5]	Dry MetHA[4,5]	NaMet[4,5]	Liq MetHA[4,5]
	Diet (%)				
Starter ration					
Corn	53.43	53.53	52.76	52.69	53.04
Soybean meal 48	38.06	37.82	38.18	38.19	38.13
Fat, animal and vegetable blend	4.63	4.58	4.85	4.87	4.75
Limestone ground	1.68	1.68	1.68	1.68	1.68
Monodicalcium PO_4	1.54	1.54	1.54	1.54	1.54
Salt	0.36	0.36	0.36	0.29	0.36
Trace minerals premix[6]	0.05	0.05	0.05	0.05	0.05
Vitamin Premix[7]	0.25	0.25	0.25	0.25	0.25
Supplemental Met	0	0.18	0.34	0.44	0.20
Grower ration					
Corn	61.42	62.01	61.71	61.69	61.82
Soybean meal 48	30.47	30.28	30.42	30.43	30.40
Fat, animal and vegetable blend	4.43	4.23	4.34	4.34	4.30
Limestone ground	1.45	1.65	1.65	1.65	1.65
Monodicalcium PO_4	1.59	1.11	1.11	1.11	1.11
Salt	0.34	0.34	0.34	0.31	0.34
Trace minerals premix[6]	0.05	0.05	0.05	0.05	0.05
Vitamin premix[7]	0.25	0.25	0.25	0.25	0.25
Supplemental Met	0	0.07	0.13	0.17	0.08

[1]Diet contained 3,135 kcal of ME/kg and 23% CP.

[2]Diet contained 3,200 kcal of ME/kg and 20% CP.

[3]Total Met activity in control diet was 0.35%.

[4]Total Met activity in the starter ration was 0.50%. Dry MetHA = dry Met hydroxy analogue; NaMet = sodium methioninate aqueous solution; Liq MetHA = liquid Met hydroxy analogue.

[5]Total Met activity in grower ration was 0.38%.

[6]Trace mineral premix added at this rate yielded 149.6 mg of Mn, 125.4 mg of Zn, 16.5 mg of Fe, 1.7 mg of Cu, 1.05 mg of I, 0.25 mg of Se, a minimum of 6.27 mg of Ca, and a maximum of 8.69 mg of Ca/kg of diet. The carrier was Ca carbonate, and the premix contains less than 1% mineral oil.

[7]Vitamin premix added at this rate yielded 11,023 IU of vitamin A, 3,858 IU of vitamin D, 46 IU of vitamin E, 0.0165 mg of B_{12}, 5.845 mg of riboflavin, 45.93 mg of niacin, 20.21 mg of D-pantothenic acid, 477.67 mg of choline, 1.47 mg of menadione, 1.75 mg of folic acid, 7.17 mg of pyroxidine, 2.94 mg of thiamin, and 0.55 mg of biotin/kg of diet. The carrier was ground rice hulls.

TABLE 4. Broiler body weights for experiments 1 and 2 at wk 6

Treatment group[1]	Experiment 1 BW[2] (g)	Experiment 2 BW[2] (g)	Experiment 2 Broiler excreta moisture[3] (%)
Control	1,937	2,080	81.86
D,L-Met	1,956	2,088	80.40
Dry MetHA	1,985	2,119	82.76
NaMet	1,838	2,097	80.35
Liq MetHA	1,955	2,109	82.50
SEM[4]	70.57	58.74	1.03

[1]Control group = no supplemental Met; Dry MetHA = dry Met hydroxy analogue; Liq MetHA = liquid Met hydroxy analogue; NaMet = sodium methioninate aqueous solution.

[2]Body weights of 5 broilers per pen (3 replications) at 42 d.

[3]Total number of aluminum foil cups (6/treatment, total of 30/wk).

[4]Pooled standard error of the mean.

in order to minimize distractions while evaluating samples. The order of sample presentation to the panelist was kept consistent through each session per day. Panelists were asked not to talk about their evaluations to anyone, and they did not know their own evaluation results. This procedure helped reduce variability of sensitivity to odors between the different panelists (ISUOL, 2001).

The hydrogen sulfide analyzer was utilized to determine the concentration of hydrogen sulfide at levels from 0.001 to 50 ppm in the air above broiler excreta. The instrument's sensitivity was given as 0.003 ppm with a reported precision of 5% relative standard deviation (ISUOL, 2001).

Statistical Analysis

Bird weight, excreta moisture, volatile sulfur compound concentrations, electronic nose individual sensor readings, and odor detection threshold data for both experiments were subjected to ANOVA using the GLM procedure of SAS software.[10] Mean differences were separated by the probability of difference (PDIFF) option (pair-wise *t*-tests). Statistical significance for all data was considered at $P < 0.05$.

RESULTS AND DISCUSSION

There were no significant differences in broiler BW and excreta moisture among the treatments in either experiment (Table 4). These data were collected to verify that broilers for all treatment groups were reared under the same conditions and that the diets had no effect on growth. Differences in BW and excreta moisture content may cause differences in manure composition and production.

The GC/MS-FDP analyses of broiler excreta were able to identify and quantify the following in the poultry fecal matter: 1) hydrogen sulfide, 2) carbonyl sulfide, 3) methyl mercaptan, 4) dimethyl disulfide, and 5) dimethyl trisulfide (Figure 1). It has been reported that hydrogen sulfide and methyl mercaptan are 2 of the 10 most odorous compounds found in livestock waste (O'Neill and Phillips, 1992). These researchers also concluded that odors that cause nuisances have been described as a sulfury smell.

Quantification of Volatile Sulfur Compounds in Poultry Feces

The concentrations of hydrogen sulfide for NaMet, control, dry MetHA, Liq MetHA, and D,L-Met treatment groups were 224.4, 49.6, 31.5, 28.6, and 26.6 ng/g feces, respectively (Table 5). The NaMet group was significantly higher than the other 4 treatment groups. The NaMet group ranged from 200 to 170 ng/g greater concentration than in the other treatment groups. The control group was significantly higher than dry MetHA, Liq MetHA, and D,L-Met treatment groups. The dry MetHA

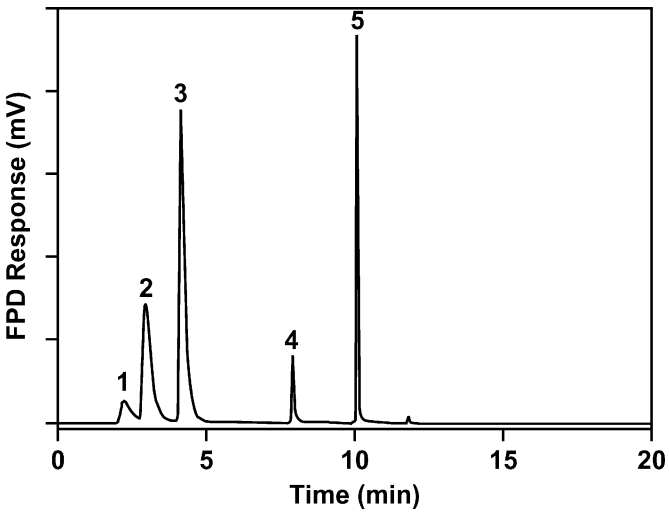

FIGURE 1. Gas chromatography with sulfur selective flame photometric detection (GC/FDP) trace of volatile sulfur compounds in broiler excreta. The peak retention times are: 1) 2.23 min, H_2S; 2) 2.95 min, carbonyl sulfide, (COS); 3) 4.14 min, methyl mercaptan, (CH_3SH); 4) 7.90 min, dimethyl disulfide, (CH_3SSCH_3); and 5) 10.06 min, dimethyl trisulfide, (CH_3SSSCH_3).

[10]SAS User's Guide, 1999, SAS Institute Inc., Cary, NC.

TABLE 5. Gas chromatography/mass spectrometry (GC/MS) determination of sulfur compound concentrations[1] of broiler excreta for experiment 1 at wk 6

	Analyte concentration in feces (ng/g)				
Treatment group[2]	Hydrogen sulfide	Carbonyl sulfide	Methyl mercaptan	Dimethyl disulfide	Dimethyl trisulfide
Control	49.6[b]	37.4[b]	9.4[d]	5.6[c]	2.9[d]
D,L-Met	26.6[d]	30.6[c]	36.1[b]	6.3[b]	7.8[a]
Dry MetHA	31.5[c]	43.9[b]	41.7[a]	6.8[b]	5.6[b]
NaMet	224.4[a]	176.1[a]	29.2[c]	11.5[a]	3.6[c]
Liq MetHA	28.6[d]	28.9[c]	10.7[d]	5.7[c]	5.2[b]
SEM[3]	1.64	1.86	0.71	0.10	0.13

[a–d]Means within columns with no common superscript differ significantly ($P < 0.05$).

[1]Total number of GC/MS analyses (3 injections per treatment).

[2]Control group = no supplemental Met; Dry MetHA = dry Met hydroxy analogue; Liq MetHA = liquid Met hydroxy analogue; NaMet = sodium methioninate aqueous solution.

[3]Pooled standard error of the mean.

group was also significantly higher than Liq MetHA and D,L-Met. There were no statistical differences between the Liq MetHA and D,L-Met groups. These 2 treatment groups had the lowest concentration of hydrogen sulfide.

Carbonyl sulfide concentrations for NaMet, dry MetHA, control, D,L-Met, and Liq MetHA were 176.1, 43.9, 37.4, 30.6, and 28.9 ng/g feces, respectively (Table 5). Again, NaMet was significantly higher than in the other 4 treatment groups. The NaMet concentration of

TABLE 6. Electronic nose[1] sensor readings of broiler excreta for experiment 2 at wk 6

	Treatment group[2]					
Sensor	Control	D,L-Met	Dry MetHA	NaMet	Liq MetHA	SEM[3]
			[(R′-R/R[4]) × 100]			
S 1	0.00062[c]	0.00107[ab]	0.00105[ab]	0.00101[b]	0.00130[a]	0.00011
S 2	0.00033[b]	0.00062[a]	0.00068[a]	0.00068[a]	0.00082[a]	0.00007
S 3	0.00050[c]	0.00081[b]	0.00088[ab]	0.00089[ab]	0.00108[a]	0.00009
S 4	0.00058[b]	0.00095[a]	0.00096[a]	0.00096[a]	0.00110[a]	0.00010
S 5	0.00880[b]	0.01250[a]	0.01230[ab]	0.01230[ab]	0.01580[a]	0.00129
S 6	0.00250[b]	0.00510[a]	0.00450[ab]	0.00430[ab]	0.00530[a]	0.00076
S 7	0.00048[b]	0.00065[ab]	0.00068[a]	0.00066[a]	0.00081[a]	0.00006
S 8	0.00050[c]	0.00081[b]	0.00082[b]	0.00084[ab]	0.00100[a]	0.00008
S 9	0.00120[b]	0.00203[a]	0.00208[a]	0.00206[a]	0.00250[a]	0.00020
S 10	0.00049[b]	0.00083[a]	0.00079[a]	0.00079[a]	0.00101[a]	0.00009
S 11	0.00070[b]	0.00100[a]	0.00100[ab]	0.00100[ab]	0.00130[a]	0.00012
S 12	0.00067[c]	0.00120[b]	0.00120[ab]	0.00120[ab]	0.00150[a]	0.00013
S 13	0.00035[b]	0.00060[a]	0.00061[a]	0.00061[a]	0.00074[a]	0.00005
S 14	0.00047[b]	0.00079[a]	0.00079[a]	0.00080[a]	0.00100[a]	0.00007
S 15	0.00100[b]	0.00140[a]	0.00140[a]	0.00140[a]	0.00180[a]	0.00014
S 16	0.00058[b]	0.00089[a]	0.00096[a]	0.00091[a]	0.00110[a]	0.00009
S 17	0.00099[b]	0.00140[a]	0.00140[a]	0.00140[a]	0.00170[a]	0.00013
S 18	0.00110[b]	0.00180[a]	0.00180[a]	0.00180[a]	0.00230[a]	0.00019
S 19	0.00031[c]	0.00053[b]	0.00059[ab]	0.00058[ab]	0.00074[a]	0.00006
S 20	0.00110[b]	0.00190[a]	0.00200[a]	0.00190[a]	0.00240[a]	0.00020
S 21	0.00085[b]	0.00120[a]	0.00120[a]	0.00120[a]	0.00150[a]	0.00013
S 22	0.00057[b]	0.00083[a]	0.00083[a]	0.00081[a]	0.00098[a]	0.00008
S 23	0.00130[b]	0.00190[a]	0.00190[a]	0.00190[ab]	0.00230[a]	0.00019
S 24	0.00017[b]	0.00047[a]	0.00043[a]	0.00043[a]	0.00060[a]	0.00006
S 25	0.00047[b]	0.00083[a]	0.00084[a]	0.00085[a]	0.00100[a]	0.00008
S 26	0.00093[c]	0.00160[b]	0.00170[ab]	0.00170[ab]	0.00210[a]	0.00018
S 27	0.00040[c]	0.00071[b]	0.00080[ab]	0.00078[ab]	0.0010[a]	0.00007
S 28	0.00160[b]	0.00260[a]	0.00260[a]	0.00260[a]	0.00330[a]	0.00027
S 29	0.00130[b]	0.00220[a]	0.00230[a]	0.00230[a]	0.00280[a]	0.00023
S 30	0.00051[b]	0.00092[a]	0.00093[a]	0.00093[a]	0.00110[a]	0.00009
S 31	0.00550[b]	0.00720[ab]	0.00690[ab]	0.00680[ab]	0.00850[a]	0.00069
S 32	0.00033[b]	0.00066[a]	0.00069[a]	0.00069[a]	0.00086[a]	0.00007

[a–c]Values within a row with different superscripts differ significantly ($P < 0.05$).

[1]Cyranose 320, Cyranose Sciences, Inc., Pasadena, CA.

[2]Control group = no supplemental Met; Dry MetHA = dry Met hydroxy analogue; Liq MetHA = liquid Met hydroxy analogue; NaMet = sodium methioninate aqueous solution.

[3]Pooled standard error of the mean.

[4]Average of 15 air sample readings for each treatment group; R′ = resistance of sensor at any time during test, and R = resistance of sensor at the start of the test.

TABLE 7. Detection threshold of odor in broiler excreta by olfactometry laboratory[1] for experiment 2 at wk 6

	Odor sample group[2]				
Sample concentration	Control	D,L-Met	Dry MetHA	NaMet	Liq MetHA
(odor units)	350[b]	526[a]	568[a]	493[a]	496[a]
SEM[3]	41.84	41.84	41.84	41.84	41.84

[a,b]Means within rows with no common superscript differ significantly ($P < 0.05$).

[1]Air samples (3 per treatment, a set minimum of 8 L were sampled in 10-L Tedlar odor sample bag) were evaluated by a minimum of 8 trained panelists (Iowa State University Olfactometry Laboratory) for odor detection threshold.

[2]Control group = no supplemental Met; Dry MetHA = dry Met hydroxy analogue; Liq MetHA = liquid Met hydroxy analogue; NaMet = sodium methioninate aqueous solution.

[3]Pooled standard error of the mean.

carbonyl sulfide ranged from 130 to 150 ng/g higher than in the other treatment groups. The dry MetHA group and the control were significantly higher than in the Liq MetHA and D,L-Met groups. There were no statistical differences between Liq MetHA and D,L-Met groups. Again, these 2 treatment groups had the lowest concentration for this sulfur compound.

Concentrations of methyl mercaptan in the dry MetHA, D,L-Met, NaMet, Liq MetHA, and control groups were 41.7, 36.1, 29.2, 10.7, and 9.4 ng/g feces, respectively (Table 5). For methyl mercaptan, dry MetHA was significantly higher than the other 4 Met treatment groups. Analyte concentration of dry MetHA ranged from 5 to 30 ng/g higher than remaining treatment groups. In addition, D,L-Met was significantly greater than in the other 3 treatment groups. The NaMet group was also significantly greater in concentration than Liq MetHA and the control group. The Liq MetHA and control groups had the lowest concentrations.

Analyte concentration of dimethyl disulfide for NaMet, dry MetHA, D, L-Met, Liq MetHA, and control were 11.5, 6.8, 6.3, 5.7, and 5.6 ng/g feces, respectively (Table 5). The NaMet treatment group had the highest concentration, which was significantly higher than the other 4 treatment groups. The dry MetHA and D,L-Met groups were significantly greater than Liq MetHA and control group.

The concentration of dimethyl trisulfide for D,L-Met, dry MetHA, Liq MetHA, NaMet, and control were 7.8, 5.6, 5.2, 3.6, and 2.9 ng/g feces, respectively (Table 5). The D,L-Met group had the highest concentration of dimethyl trisulfide. It was also significantly higher than the remaining treatment groups. There were no differences between dry MetHA and Liq MetHA groups. These 2 treatment groups were higher than NaMet and control Met treatment groups. The control group was significantly lower than the NaMet group.

For all 5 volatile sulfur compounds identified and quantified by GC/MS in the broiler excreta, the control group never had the highest concentration for experiment 1 indicating that the 4 supplemental Met sources may play a role in odorant production in broiler excreta. This finding is very important because previous research (Chavez et al., 2001) in our laboratory indicated differences in odor volatiles in broiler excreta of different supplemental Met sources as detected by electronic nose readings. However, these data could not indicate specific difference in individual compounds within the broiler excreta (Chavez et al., 2001). With the use of GC/MS it is clear that supplemental Met sources actually result in differences in volatile sulfur compounds in broiler excreta.

Electronic Nose Study

The electronic nose readings at wk 6 (experiment 2) are the only electronic nose readings included in this study. Electronic nose readings ranging from wk 2 to 6 were reported previously (Chavez et al., 2001).

TABLE 8. Hydrogen sulfide concentrations of broiler excreta by the Arizona instrument hydrogen sulfide analyzer[1] for experiment 2 at wk 6

	Odor sample group[2]				
	Control	D,L-Met	Dry MetHA	NaMet	Liq MetHA
Sample concentration (ppm)	0.005	0.009	0.008	0.006	0.008
SEM[3]	0.001	0.001	0.001	0.001	0.001

[1]Air samples (3 per treatment, reading taken for 30 seconds) were analyzed for hydrogen sulfide concentrations by a Jerome Hydrogen Sulfide Analyzer (Model 631-X, Arizona Instrument, Tempe, AZ).

[2]Control group = no supplemental Met; Dry MetHA = dry Met hydroxy analogue; Liq MetHA = liquid Met hydroxy analogue; NaMet = sodium methioninate aqueous solution.

[3]Pooled standard error of the mean.

The electronic nose data readings are shown in Table 6. For 19 sensors (2, 3, 9,10, 13 to 18, 20 to 22, 24, 25, 28 to 30, and 32), the 4 supplemental Met treatment groups (D,L-Met, dry MetHA, NaMet, and Liq MetHA) were not significantly different from each other. However, the 4 treatment groups did have electronic nose readings significantly higher than the control group. For the other 13 sensors (1, 4 to 8, 11, 12, 19, 23, 26, 27, 31), there were significant differences among all treatment groups. The control group never had the highest electronic nose readings. Persaud et al. (1996) indicated that the intensity of the signal (electronic nose readings) is proportional to the concentration of the volatile presented to the sensor. This finding indicates that, in the present study, the control group did not produce as much odor volatiles as the treatment groups.

Olfactometry Laboratory Odor Evaluation Procedure

The odor detection threshold data of the sensory panel compare well with the electronic nose readings in experiment 2. The odor detection thresholds of dry MetHA, D,L-Met, Liq MetHA, NaMet, and control were 568, 526, 496, 493, and 350 odor units, respectively (Table 7). The odor detection thresholds for the 4 supplemental Met treatment groups (dry MetHA, D,L-Met, Liq MetHA, and NaMet) were significantly higher than for the control group, which suggests that the 4 Met supplemental treatments had more odorant production than the control group. Analysis of hydrogen sulfide concentrations by the Jerome hydrogen sulfide analyzer for the control, dry MetHA, D,L-Met, Liq MetHA, and NaMet were 0.005, 0.008, 0.009, 0.008, and 0.006 ppm, respectively (Table 8). There were no statistical differences in hydrogen sulfide concentrations of broiler excreta obtained by the Jerome hydrogen sulfide analyzer for all treatment groups, whereas the GC/MS indicated differences in hydrogen sulfide concentrations (Table 5). The difference may be attributed to 1) the finding that the odorous sulfur compounds emitted from livestock waste are unstable and very reactive and are quickly oxidized (Spolestra, 1980; O'Neill and Phillips, 1992) or 2) that the partitioning equilibrium for hydrogen sulfide, and other acidic sulfur compounds, may be affected by the treatment. These effects may include treatment-induced changes in the bulk pH of the excreta or changes in the microenvironment of the excreta due to presence of ion exchanging compounds (e.g., amines and phenolics). The extraction procedures used in the GC/MS analysis provided normalized conditions in which the matrix (excreta) has little influence on the measured analyte concentration. However, because these extraction procedures are not used with the Jerome meter, this method is susceptible to complex matrix interferences.

The analysis of the excreta by GC/MS was able to show that there were statistically significant differences among the Met treatment groups in 5 volatile sulfur compound concentrations. The control group had the lowest concentration for any of the 5 volatile sulfur compounds. The majority of the electronic nose sensors (19 of 32) indicated that the 4 supplemental treatment groups had significantly higher readings than the control group. The odor detection threshold of the 4 supplemental treatment groups had higher odor detection thresholds than the control group. These measurements of odor volatile concentration in broiler excreta demonstrate that the 4 supplemental treatment groups do play a role in odor production.

The primary aim of this study was to determine if supplemental dietary Met sources play a role in volatile odor production and the odorous chemical concentration in broiler excreta. Both experiments demonstrated that supplemental Met plays a role in volatile odor production in excreta. However, these data do not indicate that the 4 supplemental Met treatment groups contribute to odor offensiveness of broiler excreta. In a future study, broiler excreta will be analyzed by a trained human descriptive aroma attribute sensory panel to determine offensiveness.

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